Metabolism of Morphine by Cats

S. Y. YEH, H. I. CHERNOV*, and L. A. WOODS†

Abstract □ Cats excreted 19.2 ± 1.5% of free morphine and 48.4 ± 2.4% of conjugated morphine in urine during the first 48 hr. after subcutaneous injection of morphine. Fecal excretion during 2 days following drug administration ranged from 2.0 to 20% of the injected drug. The major morphine conjugate was isolated and characterized as morphine-3-ethereal sulfate by UV and IR spectra, morphine content, and elemental analysis. In addition to morphine-3-ethereal sulfate, morphine-3-glucuronide and normorphine were also identified as minor metabolites.

Keyphrases □ Morphine—metabolism, metabolites, cats □ Metabolites, urinary, fecal—morphine, cats □ IR spectrophotometry—analysis □ UV spectrophotometry—analysis

One of the morphine conjugates excreted by cats was isolated and characterized as morphine ethereal sulfate (1, 2). The present article describes the isolation and characterization of this and other morphine metabolites and gives data on the urinary and fecal excretion of free morphine and conjugated morphine up to 48 hr. following drug administration.

METHODS

Animal Experiments—Domestic male and female cats, weighing 1.4–3.0 kg., were injected subcutaneously with 20 mg./kg. (as the base) of *N*-$^{14}CH_3$-morphine HCl. Urine and feces were collected daily in a metabolism pan for 48 hr. (at least two samples collected). The pan was thoroughly washed after each collection, and the pooled urine and washings were frozen immediately.

Three cats were sacrificed 3 hr. after drug administration. The animals were given an intrathoracic injection of 50 mg./kg. of sodium pentobarbital followed by exsanguination *via* the carotid artery. Bile was obtained by aspiration.

Determination of Total Radioactivity, Free Morphine, and Conjugated Morphine—Free morphine, conjugated morphine, and total radioactivity in the urine were determined by the procedure described previously (3).

Isolation and Purification of the Major Morphine Conjugate from Cats—The urine sample (450 ml.) was loaded on 10 g. of acid-washed and activated charcoal[1]. The column was washed with 100 ml. of water. Morphine and its metabolites were either eluted with 350 ml. of 50% acetic acid in water or 400 ml. of ammoniacal (30% NH_3) methanol (1:1) from the column, and the eluate was evaporated to dryness at 50° under reduced pressure. The residue was triturated with methanol and crystallized from about 50 ml. of boiling water. After refrigeration overnight the colorless needle-shaped crystals were filtered, washed with methanol, and dried at 60° *in vacuo* for 8 hr. The yield of morphine conjugate from several experiments was 110 mg., 35% of injected morphine.

The methanolic supernatant was evaporated to dryness, and the residue was dissolved in a small amount of dilute HCl, adjusted to pH 10 with NH_4OH, saturated with NaCl, and extracted with ethylene chloride containing 30% of *n*-amyl alcohol. The extract was evaporated to dryness under reduced pressure. Chromatography of the residue, dissolved in methanol on Whatman 3 MM paper buffered with citrate–sodium phosphate solution at pH 6.8 (4) and developed with *n*-butanol and water (9:1), showed two spots having R_f values of 0.12 (normorphine) and 0.66 (morphine) after spraying with potassium iodoplatinate solution or 3% ninhydrin in methanol.

[1] 50–200 mesh, Fisher Scientific Co., Fair Lawn, N. J.

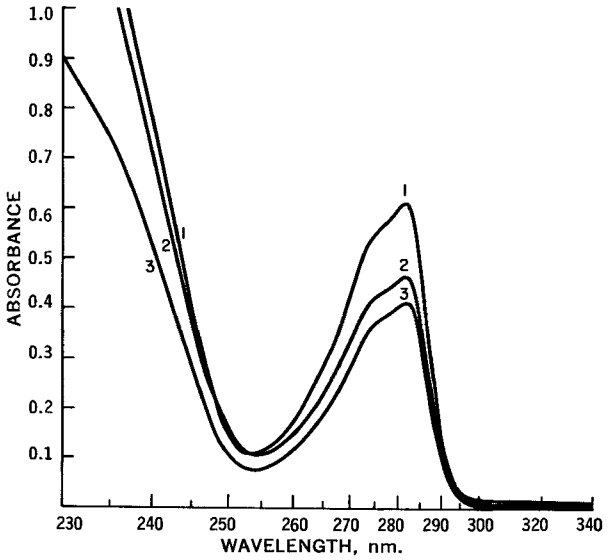

Figure 1—*UV spectrum of morphine conjugate isolated from urine of cats (morphine-3-ethereal sulfate). Key: 1, H_2O; 2, NaOH; and 3, HCl.*

Synthesis of Morphine Ethereal Sulfate—Morphine-3-ethereal sulfate was synthesized according to the procedure of Stolnikow (5).

RESULTS

Urinary, Fecal, and Biliary Excretion of Morphine by Cats—Urinary excretion of free morphine and conjugated morphine by nine cats after subcutaneous injection of morphine was 19.2 ± 1.5% and 48.4 ± 2.4%, respectively, of injected dose.

Fecal excretion of total morphine from six out of nine cats during 2 days following drug administration ranged from 2.0 to 20% of the injected dose, 2.1 ± 0.41% being free morphine and 8.3 ± 2.54% being conjugated morphine. One percent of the injected radioactivity was found in the 2–6 day feces of three animals which did not defecate within 48 hr.

Total drug excreted in the urine and feces was about 80% of the injected dose.

Recoveries of total morphine in the bile 3 hr. after subcutaneous injection of 20 mg./kg. of morphine from three cats were 22.7, 14.5, and 5.1% of the injected dose. The ratios of conjugated to free morphine were 5.7, 4.0, and 2.1.

Detection of Morphine Metabolites from Cats—The paper chromatograms of (*a*) the cat urine and (*b*) the eluate from the charcoal column on Whatman 3 MM paper, developed with *n*-butanol–acetic acid–water (4:1:2), showed two spots having R_f values of 0.42 (major metabolite, later identified as morphine-3-ethereal sulfate) and 0.70 (morphine). The mother liquor, after crystallization of the major morphine conjugate from the concentrated eluate, showed three spots having R_f values of 0.29 (morphine-3-glucuronide), 0.42, and

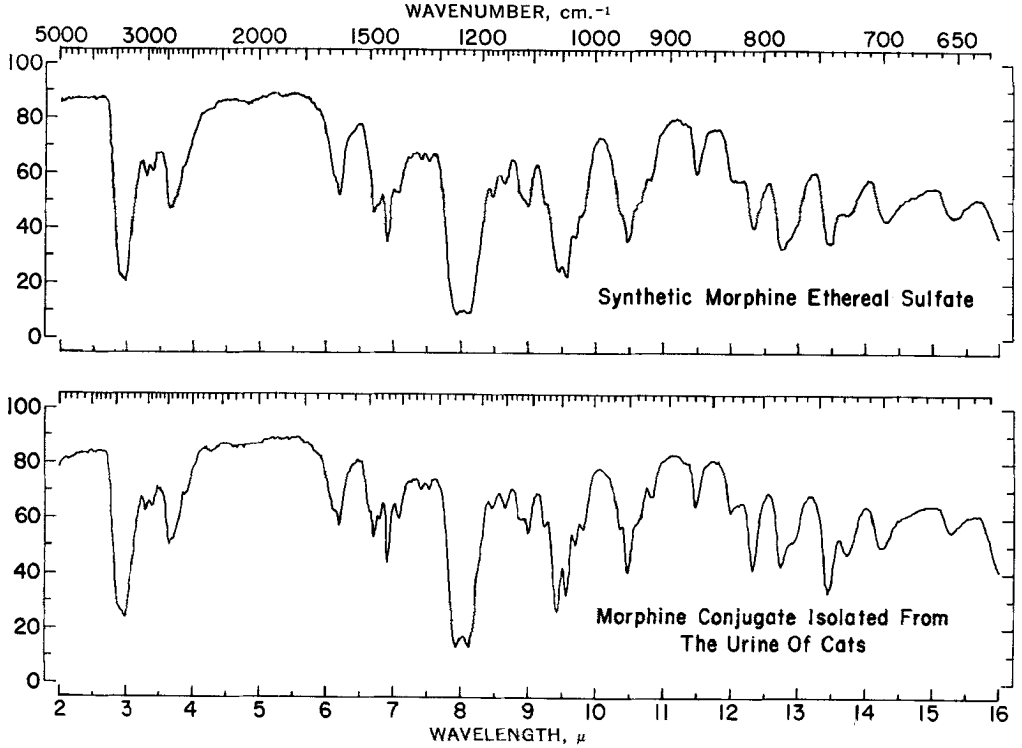

Figure 2—*IR spectra of morphine-3-ethereal sulfate.*

0.70. Normorphine was detected in the extract from the mother liquor after crystallization of morphine conjugate from the eluate, as described in the *Method* section.

Acid hydrolysis of the metabolite gave morphine, detected by the paper chromatography, indicating that the metabolite was a conugated morphine.

The results of incubation of 1-ml. aliquots of the urine or the isolated morphine conjugate solution (buffered with 3 ml. of 0.2 *M* sodium acetate at pH 5.0) with 15 mg. (60 units) of limpet phenol sulfatase[2], 0.1 ml. of glusulase[3], 0.2 ml. of ketodase[4], or 37.5 mg. (800 units) of β-glucuronidase[2] (buffered with 3 ml. of 0.2 *M* phosphate at pH 7.0) for 20 hr. in air showed that the morphine conjugate in the urine was hydrolyzed with glusulase but not with limpet phenol sulfatase and β-glucuronidase. The isolated morphine conjugate was completely hydrolyzed with glusulase and phenol sulfatase, but not by ketodase and β-glucuronidase. The results indicated that the isolated morphine conjugate contained a sulfate group.

Characterization of the Major Morphine Metabolite from Cats—The metabolite is insoluble in water, dilute acid solution, and organic solvents; it is sparingly soluble in hot water and soluble in alkaline solution. The metabolite does not melt nor discolor at 300° and does not show the typical morphine phenolic reaction after sequential treatment with alkaline ferricyanide and acid chloride solution.

The UV spectrum of the metabolite in NaOH solution did not show bathochromic shift (*a*, 285 nm. ≃6.0) (Fig. 1).

The IR spectrum of the metabolite as a pressed KBr pellet showed strong absorption bands at 1240–1280 cm.$^{-1}$, indicating that the metabolite contained a sulfate group. Presence of an absorption band at 2600–2860 cm.$^{-1}$ indicates the tertiary amine salt formation and suggests that the compound exists as a zwitterion. Absorption in the 3600-cm.$^{-1}$ region apparently is due to the secondary alcohol group (Fig. 2).

The morphine conjugate isolated from cats was identical with the synthetic morphine ethereal sulfate as shown by the IR and UV spectra, morphine content (76.8%) determination, phenolic test, and elemental analysis[5].

Anal.—Calcd. for $C_{17}H_{19}NO_6S$: C, 55.8; H, 5.24; N, 3.83; S, 8.78. Found: C, 55.46; H, 5.33; N, 3.84; S, 8.42.

[2] Sigma Chemical Co., St. Louis, Mo.
[3] Endo Lab., Garden City, N. Y.
[4] Warner-Chilcott Lab., Morris Plains, N. J.
[5] Huffman Lab., Wheatridge, Colo.

Data obtained in the present studies confirm the result of Fujimoto and Haarstad (2) that the major morphine metabolite in the cat is morphine-3-ethereal sulfate.

No attempt was made to isolate the minor morphine metabolites, *i.e.*, morphine-3-glucuronide (1–2% of injected dose) and normorphine detected by paper chromatography.

DISCUSSION

Urinary excretion of free morphine (19%) by cats obtained in the present studies is higher than the values (3–12% of injected dose) reported by Keeser *et al.* (6) and (6–10%) by Yoshikawa (7).

Five to twenty-three percent of the injected morphine was found in the bile of the gall bladder 3 hr. after injection. Since only 1% of the injected drug was found in the feces of three cats collected 48 hr. after injection, the data indicated that morphine and/or conjugated morphine excreted from the bile ducts was reabsorbed into the bloodstream.

In addition to morphine-3-ethereal sulfate, morphine-3-glucuronide and normorphine were detected as minor metabolites in the urine of cats injected with morphine. Detection of free normorphine as a metabolite of morphine confirms the report of Tampier and Penna-Herreros (8), who found normorphine in the cat liver homogenate 1 hr. after injection of morphine. Although morphine-3-glucuronide was detected in the mother liquor after crystallization of morphine ethereal sulfate, it was not detected in the urine with paper chromatography and the enzymatic hydrolysis method because of low concentration.

Welch *et al.* (9) reported that phenol sulfatase will completely hydrolyze phenol sulfates in 24 hr. in presence of cat urine if the urine concentration does not exceed 10%. The failure of phenol sulfatase to split off morphine ethereal sulfate in the present study may be due to a higher concentration of cat urine (25%) used for hydrolysis.

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ACKNOWLEDGMENTS AND ADDRESSES

Received July 20, 1970, from the *Department of Pharmacology' College of Medicine, University of Iowa, Iowa City, IA 52240*

Accepted for publication September 30, 1970.

This study was supported by U. S. Public Health Service Grant NB-02928.

A preliminary report of a part of these experiments appeared in: *Pharmacologist*, **8**, 206(1966); and *Fed. Proc.*, **27**, 754(1968).

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Effect of Vagal Stimulation on Duodenal Serotonin in the Guinea Pig

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Abstract □ The serotonin concentration of duodenal tissue was examined immediately after peripheral or central vagal stimulation, and an 18 or 36% reduction of serotonin, respectively, was found. This is the first demonstration of a decrease in gastrointestinal serotonin in response to autonomic nerve stimulation.

Keyphrases □ Serotonin, duodenal—effect of vagal stimulation, guinea pig □ Gastrointestinal serotonin, reduction—response to autonomic nerve stimulation □ Spectrophotofluorometry—analysis

Enterochromaffin cells (EC) are found throughout the gastrointestinal tract, but in the guinea pig their greatest concentration is in the duodenum, *juxta* the pyloric sphincter (1, 2). They are most frequently found deep in the crypts of Lieberkuhn and can be identified by staining the cell granules with silver salts after precipitation by formalin (3). These granules are believed to contain 5-hydroxytryptamine (5-HT, or serotonin), and a high degree of correlation has been reported between EC number and mucosal serotonin concentration in laboratory and domestic animals.

Stimulation of the vagi was shown to result in approximately a 75% decrease in EC counted in the proximal duodenum of the guinea pig (4). Reduction of cells counted merely reflected cellular degranulation, or at least a decrease in stainable substance within the cells. Since serotonin is most frequently identified with these cells, the purpose of this study was to determine if stimulation of the vagi is also associated with a change in tissue serotonin concentration.

EXPERIMENTAL

Animals and General Procedure—Seventy guinea pigs were secured and maintained as previously described (4). Anesthetized animals were divided into three groups. Group I animals (controls) were sacrificed in three subgroups: (*a*) immediately after achieving surgical anesthesia, (*b*) 30 min. later, or (*c*) 60 min. later. Group II animals had the vagi stimulated peripherally and were sacrificed 30 or 60 min. later. Group III animals were sacrificed at 30 or 60 min. after the initiation of central vagal stimulation. In all animals the duodenal tissue *juxta* the pyloric sphincter was removed for spectrophotofluorometric analysis of serotonin according to the method of Wise (5). One group of unanesthetized animals was sacrificed by decapitation. Of these, four received 10 mg./kg. reserpine intraperitoneally 24 hr. prior to decapitation. Results were analyzed for significance at the 0.05 level using a two-tailed Student *t* test.

Surgical and Recording Procedure—Under intraperitoneal urethan anesthesia (1.5 g./kg.), the vagi were exposed, divided, and stimulated high in the neck. At the cervical level the vagi were stimulated peripherally or centrally. Carotid arterial pressure was recorded continuously using a Statham pressure transducer. Scalar lead II of the electrocardiogram and tachograph was also recorded. All variables were charted on a polygraph (Grass model 7).

Electrical Stimulation—Bipolar electrodes were attached to the caudad or central portion of the vagi and surrounded with liquid petrolatum to isolate the stimulating current. The vagi were electrically stimulated with a Grass model S8 stimulator *via* an isolation transformer. The stimulator delivered a rectangular pulse (5 v., 30 Hz., 1 msec.), which was applied for a 5-min. duration followed by a rest interval of equal duration for a total of three times.

RESULTS

The results of this study are found in Fig. 1. The concentration of serotonin in unanesthetized animals was about 4.42 mcg./g. duodenal tissue, whereas after reserpine administration the concentration decreased to 0.43 mcg./g. or by approximately 90%.

Immediately after the guinea pigs were anesthetized, the duodenal tissue had a serotonin concentration of 3.09 mcg./g. In unstimulated controls the slight average increase to 3.29 mcg./g. after 30 min., as well as a marginal decrease to 2.63 mcg./g. at 60 min., was not significant. Stimulation of the peripheral vagi decreased serotonin concentration to 2.48 and 2.73 mcg./g. at 30 and 60 min., respectively. The 30-min. concentration was significantly different from both prestimulation and 30-min. controls, but at 60 min. this change was not significant. Central vagal stimulation decreased duodenal serotonin to 1.97 mcg./g. at 30 min. and 2.29 mcg./g. at 60 min. Both concentrations were significantly different with respect to prestimulation control, but only the 30-min. sample was statistically different from its respective control.

DISCUSSION

Pentilla (6) demonstrated a significant correlation between the number of EC cells and duodenal serotonin concentration in the mouse, rat, guinea pig, rabbit, sheep, pig, cow, and horse. The regression correlation coefficient of his study was determined on interspecies data, but the duodenal serotonin data from any one species generally did not appear to reflect this correlation. However, there was a modest correlation of EC and serotonin concentration in the rat duodenum during the first 2 months of life (6), and a strong correlation for developing chicken embryonic duodenum (7). Even more significantly, Pentilla (8) reported serotonin concentra-